IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

DOCKET NUMBER	ANTICIPATED CLASSIFICATION OF THIS APPLICATION:		PRIOR APPLICATION	
	CLASS	SUBCLASS	EXAMINER	ART UNIT
8076.102USC1			S. PRIEBE	1632

CERTIFICATE UNDER 37 CFR 1.10
"Express Mail" mailing label number: EL119857291US
Date of Deposit. DECEMBER 3, 1998

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By _____
Name: T. Bost

CONTINUATION APPLICATION UNDER 37 C.F.R. § 1.53(b)

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, DC 20231

Dear Sir:

This is a request for filing a continuation application under 37 CFR § 1.53(b) of Serial No. 08/619,157, filed on MARCH 21, 1996 entitled DEFECTIVE RECOMBINANT ADENOVIRUSES EXPRESSING CYTOKINES FOR USE IN ANTITUMORAL TREATMENT by the following inventor(s):

Full Name Of Inventor	**Family Name** HADDADA	**First Given Name** HEDI	**Second Given Name**
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Full Name Of Inventor	**Family Name** RAGOT	**First Given Name** THIERRY	**Second Given Name**
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Full Name Of Inventor	**Family Name** PERRICAUDET	**First Given Name** MICHEL	**Second Given Name**
Residence & Citizenship	**City** OUARVILLE	**State or Foreign Country** FRANCE	**Country of Citizenship** FRANCE
Post Office Address	**Post Office Address** 20, RESIDENCE DU MOULIN	**City** OUARVILLE	**State & Zip Code/Country** F-28150 / FRANCE

1. ☒ Enclosed is a copy of the prior application; including the specification, claims, drawings, oath or declaration showing the applicant's signature, and any amendments referred to in the oath or declaration filed to complete the prior application. (It is noted that no amendments referred to in the oath or declaration filed to complete the prior application introduced new matter therein.) The continuing application is as follows: 15 pages of specification, 13 claims, 1 page of abstract, 1 sheets of drawings, and 2 pages of oath or declaration.

☒ The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

2. ☒ Cancel original claims 2, and 9–13 of this application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

3. ☒ The filing fee is calculated below:

CLAIMS AS FILED

NUMBER FILED	NUMBER EXTRA		RATE	FEE
TOTAL CLAIMS: 07 -20	00	x	$018.00	$0.00
INDEPENDENT CLAIMS 02 -3	00	x	$078.00	$0.00
			BASIC FILING FEE:	$0760.00
			TOTAL FILING FEE:	$760.00

☐ A Verified Statement that this filing is by a small entity is already filed in the prior application.

☐ A Verified Statement that this filing is by a small entity is attached.

4. ☒ Payment of fees:
   ☒ Attached is a check in the amount of $760.00.
   ☐ Please charge Deposit Account No. 13–2725.

5. ☒ The Commissioner is hereby authorized to charge any additional fees as set forth in 37 CFR §§ 1.16 to 1.18 which may be required by this paper or credit any overpayment to Account No. 13–2725.

6. ☒ Amend the specification by inserting before the first line the sentence:

"This application is a Continuation of application Serial No. 08/619,157, filed March 21, 1996, which application(s) are incorporated herein by reference."

7. ☐ A set of formal drawings (_____ sheets) is enclosed.

8. ☒ Priority of application Serial No. 9203120, filed on March 16, 1992 in France, is claimed under 35 U.S.C. 119.

☐ The certified copy has been filed in prior application Serial No. _____, filed _____.

9. ☒ The prior application is assigned of record to CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE INSTITUT GUSTAVE ROUSSY located at PARIS, FRANCE.

10. ☒ The Power of Attorney in the prior application is to:

Merchant, Gould, Smith, Edell,
Welter & Schmidt, P.A.
3100 Norwest Center
90 South Seventh Street
Minneapolis, MN 55402–4131

11. ☒ A preliminary amendment is enclosed. (Claims added by this amendment have been properly numbered consecutively beginning with the number next following the highest numbered original claim in the prior application.)

☐ Fee for excess claims is attached.

12. ☒ A petition and fee has been filed to extend the term in the prior application until December 3, 1998. A copy of the petition for extension of time in the prior application is attached.

13. ☐ The inventor(s) in this application are less than those named in the prior application and it is requested that the following inventors identified above for the prior application be deleted:

14. ☒ Also Enclosed: Declaration of Majid Mehtali

15. ☒ Address all future communications to the **Attention of Mark T. Skoog** (may only be completed by attorney or agent of record) at the address below.

16. ☒ A return postcard is enclosed.

Respectfully submitted,

MERCHANT, GOULD, SMITH, EDELL
WELTER & SCHMIDT, P.A.
3100 Norwest Center
90 South Seventh Street
Minneapolis, Minnesota 55402
(612) 332-5300

Date: Dec 3, 1998

Mark T. Skoog

Mark T. Skoog
Reg. No. 40,178
MTS:PSTlls

S/N FILED HEREWITH PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	HADDADA ET AL.	Examiner:	UNKNOWN
Serial No.:	FILED HEREWITH	Group Art Unit:	UNKNOWN
Filed:	FILED HEREWITH	Docket No.:	8076.102US~~01~~C1
Title:	DEFECTIVE RECOMBINANT ADENOVIRUSES EXPRESSING CYTOKINES FOR USE IN ANTITUMORAL TREATMENT		

CERTIFICATE UNDER 37 CFR 1.10:
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By: Tim Just
Name: T. Just

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to examination on the merits Applicants offer the following amendments and remarks.

In the Claims

1. (Amended) [Recombinant nucleic acid containing, on the one hand] An adenoviral vector comprising a genomic sequence of an adenovirus [which] wherein said genomic sequence:

(a) is defective in that [it] said adenovirus lacks [the sequences] a sequence needed for its replication, but which [nevertheless] contains those sequences which[, in this genome, are the carrier of the] carry genetic information needed for the corresponding adenovirus to enter [the] cells [which the latter] which adenovirus is capable of infecting[, as well as the];

(b) comprises a set of essential sequences needed for encapsidation of [this] said adenovirus[,]; and [on the other hand]

(c) comprises an insert containing a nucleic acid sequence coding for a cytokine, [this] wherein said insert [being] is under [the] control of [a] an endogenous or heterologous promoter [present in or previously inserted into the above-mentioned genomic sequence.]; and

wherein said adenovirus vector lacks the transactivators E1A and E1B and the E3 region of the adenovirus.

3. (Amended) [Recombinant nucleic acid] The adenoviral vector according to claim 1, [characterized in that] wherein the genomic sequence of the adenovirus lacks its 5' end region downstream of the early promoter of the E1A region of the adenovirus, and [in that] wherein the nucleic acid sequence coding for the cytokine is placed under the control of this early promoter.

4. (Amended) [Recombinant nucleic acid] The adenoviral vector according to claim 1 [or claim 2], [characterized in that] wherein the nucleic acid sequence coding for the cytokine is placed under the control of an adenovirus late promoter.

5. (Amended) [Recombinant nucleic acid] The adenoviral vector according to claim 1, [characterized in that] wherein the genomic sequence of the adenovirus [is provided with] has heterologous a promoter [foreign to the adenovirus genome,] and [in that the] wherein said nucleic acid sequence coding for the cytokine is placed under the control of [this] said [foreign] heterologous promoter.

6. (Amended) [Recombinant nucleic acid, characterized, either in that the] An adenoviral vector comprising a nucleic acid insert [contains] containing sequences coding for several cytokines, or [in that it contains] containing separate nucleic acid inserts coding for different cytokines wherein said nucleic acid inserts are placed, [respectively,] under the control of separate promoters[, which are also separate].

7. (Amended) [Defective] A defective adenovirus, [characterized in that it contains] comprising the [recombinant nucleic acid] sequence according to [any one of claims 1 to 6] claim 1.

8. (Amended) A cell culture [Culture of cells, in particular of human origin, characterized in that they are] infected with the [adenovirus] adenoviral vector according to claim [7] 1.

REMARKS

Entry of the foregoing and favorable reexamination and reconsideration of the subject application, as amended, pursuant to and consistent with 37 C.F.R. § 1.112, and in light of the remarks which follow, are respectfully requested.

In the Advisory Action dated August 4, 1998, the Examiner maintained the rejection of claims 1 and 3–8 under 35 U.S.C. § 103(a) as being unpatentable over *Rosenfeld et al.* taken with *Russell*, *Ramshaw et al.* and *Stratford–Perricaudet et al.*

In rendering this rejection, the Examiner purports that *Rosenfeld et al.* and *Stratford–Perricaudet et al.* teach the defective adenoviral vector of the present invention which lacks the transactivators E1A and E1B and the E3 region of the adenovirus. According to the Examiner, *Russell* provides the motivation to combine cytokines with alternate viral vectors for the treatment of cancer and *Ramshaw et al.* teaches the combination of an adenoviral vector containing genes encoding lymphokines. Therefore, the Examiner asserts that a skilled artisan would search for a new viral vector system to insert a gene coding for a cytokine for use in cancer therapy by the teachings of *Russell*, arrive at the teachings of *Ramshaw et al.* wherein the combination of an adenoviral vector and cytokine is taught and modify that vector to delete the E1 region.

However, Applicants submit that the Examiner's reliance on the primary reference of *Rosenfeld et al.* could only be through hindsight. To select among all of the vectors known in the art as of the filing date of the present invention and deem that an adenoviral vector would work could only be accomplished with the Applicants' claims in mind. Applicants further submit that a skilled artisan would start with the teachings of *Russell*, which suggests the use of other vectors in conjunction with a cytokine for cancer therapy, then proceed to *Ramshaw et al.* which teaches a variety of viral vectors containing a nucleotide sequence encoding a cytokine and then further proceed to alter this vector via the teachings of *Rosenfeld et al.* for cancer therapy since *Stratford–Perricaudet et al.* teaches many uses for these vectors.

However, Applicants submit that there is simply no guidance nor any expectation of success given to the skilled artisan to choose the presently claimed vector from those known in the art to treat tumors *in vivo*. Indeed, the problems associated with treating tumors are different

from the problems associated with treating a gene deficiency as will be discussed more extensively below.

First of all, it is well known in the case law that to prove a *prima facie* case of obviousness, not only a suggestion to modify is required in the combination of prior art references, but also there must be some suggestion in the combination of the prior art references of an expectation of success or the desirability of the combination. See, *In re Dow Chemical Co.*, 837 F.2d 469, 5 USPQ2d 1529 (Fed. Cir. 1988) and *Also Standard Corp. v. Tennessee Valley Authority*, 808 F.2d 1490, 1 USPQ2d 1337 (Fed. Cir. 1986). This is clear from *In re Dow Chemical Co., supra* where the court stated the following:

> The consistent criterion for determination of obviousness is whether the prior art would have suggested to one of ordinary skill in the art that this process should be carried out and would have a reasonable likelihood of success, viewed in light of the prior art . . . Both suggestion and the expectation of success must be founded in the prior art, not in the applicant's disclosure . . .

Therefore applying the above to *Russell*, it can be said that this reference does not suggest any expectation of success or desirablity to use defective viral vectors expressing a cytokine to treat tumors. Rather, *Russell* discourages, as Applicants have previously pointed out, the use of this type of vector and appears to encourage competent viral vectors.

It is clear in the case of *In re Dow Chemical Co., supra*, that negative teachings of the belief that the invention could not be made were also considered pertinent to the court. More specifically it was held that:

> the PTO erred in rejecting as *prima facie* obvious in view of the prior art the subject matter of the claims under reexamination relating to an impact resistant rubber–based resin having improved resistance to heat distortion; the prior art reflected a belief that the claimed products could not successfully be made by the use of the processes in the references cited as the basis for the rejection; the PTO's theory that one of ordinary skill would have found it "obvious to experiment" with the referenced processes is not a proper standard for obviousness (emphasis added).

Although *Russell* teaches the skilled artisan that genetic cytokine therapies and viral vector–mediated delivery was in the forefront, this reference also teaches that large hurdles had to be overcome prior to accomplishing this form of therapy. Furthermore, *Russell* suggests that defective viral vectors would not be successful when he clearly states:

> However, it is difficult to imagine how the problem of access to poorly vascularized tumour regions could be overcome except by the use of replication competent viruses . . .

This teaching also has to be considered and cannot be ignored, even though there is a general teaching that the feasibility of tumour targeted lymphokine gene therapy may be promising depending on whether a suitable vector can be found.

Even if a skilled artisan would look for alternative vectors that may be successful to treat tumors *in vivo* why would a skilled artisan choose an adenoviral vector when other vectors were known in the art?

For example, *Ramshaw et al.* teaches that several different types of vectors were known in the art at the critical filing date of the present invention. The disclosed vectors include vaccinia virus vectors, adenovirus vectors, poxvirus vectors, herpes virus vectors or bacterial vectors. *Ramshaw et al.* teaches in the examples, recombinant viral vectors encoding a cytokine gene using competent vaccinia virus, as well as a competent adenovirus. There is no suggestion that the adenovirus vector taught in *Ramshaw et al.* has any superior qualities over the vaccinia virus vector. In fact, all of the scientific data to illustrate that the invention works is done with the vaccinia virus.

Therefore, there is no suggestion to the skilled artisan that a competent adenoviral vector is more desirable to treat tumors *in vivo*. Nor is there any suggestion to modify the adenoviral vector of *Ramshaw et al.* to exclude the E1 region.

The Examiner relies on *Rosenfeld et al.* to deem that a skilled artisan would proceed to modify further the vector of *Ramshaw et al.* to delete the entire E1 region due to the following statement therein:

> Most human adults have antibodies to one of the three serogroup C adenoviruses to which Ad5 belongs (5). This implies little risk for those working with these vectors but may have negative implications for the virus as a gene transfer vector in the human lung. If such problems are encountered, alterations in the vector construct may be helpful.

Thus, the Examiner maintains that since it was known in the art that the E1 region is responsible for replication, the skilled artisan would delete this entire region.

Applicants disagree with the Examiner's interpretation of this teaching in *Rosenfeld et al.* This paragraph basically teaches the skilled artisan that due to the fact that most human adults have antibodies to adenoviruses, repeated administration of the vector may result in the antibodies "killing" the administered adenovirus and hence leaving the vector ineffective for therapeutic purposes, but having little risk for those working with the virus. Thus, in case the

adenovirus is "killed" by antibodies, *Rosenfeld et al.* suggests that by altering the vector construct may be helpful to solve this problem.

Altering does not necessarily mean deleting, as the Examiner suggested. Rather this definition means basically to change the adenoviral construct. However, no suggestion of how to change the construct is set forth in *Ramshaw et al.* nor *Rosenfeld et al.*

The Examiner's contention that the skilled artisan would interpret this suggestion to mean to delete the entire E1 region would in fact probably no solve the problem concerning the "killing" of the adenovirus via human antibodies, since the E1 regions, is not within the capside of the virus. Therefore, the skilled artisan would not be lead to delete the entire E1 region, as the Examiner maintains by the teachings of *Rosenfeld et al.*

Stratford–Perricaudet et al. fail to teach or suggest using an adenoviral vector to treat tumors *in vivo*. Rather, this entire reference suggests the use of adenoviral vectors to treat gene deficiencies.

Thus, the combination of references does not suggest to the skilled artisan to use the claimed defective adenoviral vectors to treat tumors *in vivo*. The only cited reference of record that teaches tumor therapy is *Russell* and this reference encourages the use of competent viral vectors and teaches away from the use of defective viral vectors.

Enclosed is a Declaration executed by Majid Mahtali that details more of the points set forth above.

Therefore, in view of the above, withdrawal of this rejection is respectfully requested.

From the foregoing, favorable action in the form of a Notice of Allowance is respectfully requested and such action is earnestly solicited.

Respectfully submitted,

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Date: Dec 3, 1998

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DEFECTIVE RECOMBINANT ADENOVIRUSES EXPRESSING CYTOKINES FOR ANTITUMOR TREATMENT

Cytokines are molecules (hormones) produced by cells following an antigenic stimulation or an activation by other factors. The first cytokine which will has been produced is interleukin-1 (Il-1). It permits activation of the T cells which, in turn, start producing a whole battery of lymphokines, some of which are essential for the activation of the immune system and the defenses against viral or parasitic infections.

For some years, cytokines have been used in anticancer immunotherapy. Nevertheless, when they are administered systemically, a number of problems arise. Il-2, for example, produces quite substantial side effects. it is rapidly metabolized, so that high doses have to be administered repeatedly.

Better administration routes which would increase their efficacy while decreasing their adverse effects are hence being sought.

The subject of the invention is hence defective recombinant adenoviruses expressing one or more cytokines, as well as the use of these recombinant adenoviruses for making up pharmaceutical compositions, in particular antitumor compositions, more especially compositions which can be injected directly into solid tumors of the host.

The subject of the present invention is defective recombinant adenoviruses, characterized in that they contain a defective, non-replicable adenovirus genome

into which one or more nucleic acid sequences coding for one more cytokines, in particular lymphokines, are inserted under the control of one or more promoters capable of being recognised by the polymerases of human cells, more especially of human tumor cells or of cells infiltrating these tumors.

The invention relates more especially to recombinant nucleic acids capable of being used for the production of such defective recombinant adenoviruses.

Such a recombinant nucleic acid is characterized in that it contains, on the one hand a genomic sequence of an adenovirus which is defective in that it lacks the sequences needed for its replication, but which nevertheless contains those sequences which, in this genome, are the carrier of the genetic information needed for the corresponding adenovirus to enter the cells which the latter is capable of infecting, as well as the set of essential sequences needed for encapsidation of this adenovirus, and on the other hand an insert containing a nucleic acid sequence coding for a cytokine, this insert being under the control of a promoter present in or previously inserted into the abovementioned genomic sequence.

Adenoviruses, in particular type 2 or 5 adenoviruses capable of infecting humans (or human adenoviruses), or alternatively serotype 4 and 7 adenoviruses, represent especially preferred vectors in the context of the present invention, on account, in particular, of the large size of the foreign DNA fragment which it is

possible to insert into the genome of these viruses.

Advantageously the abovementioned nucleic acid insertion sequence(s), coding for one or more predetermined cytokines, are contained in a defective adenovirus genome lacking the essential nucleotide sequences needed for replication of these adenoviruses, and more especially the transactivators E1A and E1B and, where appropriate, the E3 region of the adenovirus, or alternatively its E1 and E3 regions.

In other words, the invention turns to good account the capacity of these defective recombinant adenoviruses to allow the insertion sequence they contain to be expressed in the cells they invade even when, on account of their defective character, they do not multiply therein. In other words, the objective of the invention is to cause cytokines to be secreted actually within the cells of the tumour to be treated (tumor cells themselves and cells, in particular lymphocytes, which infiltrate these tumors) when these cells have been infected with these defective adenoviruses, especially when the latter are injected directly into the tumor. The cytokines produced will thus activate first and foremost, in situ, the cytotoxic cells infiltrating the tumor and those present in proximity to the tumor.

Regarding the sequence for insertion into the defective recombinant adenovirus genome, this may be chosen from all those which express a cytokine capable of exerting either a direct antitumor effect, or an activating effect on immunocompetent cells of the body, or both

together.

Among these cytokines, the following may be mentioned as examples: IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, α interferon, γ interferon, tumor necrosis factor alpha (TNFα) ([same term in French]).

The same recombinant adenoviruses may also be used in the case of certain diseases in which there is an immune deficiency and in the case of certain parasitic or viral disaeases, especially γ, α interferon and/or GM-CSF), in particular by administration systemically or via cells, preferably human cells, taken in a state that allows them to be injected into humans, these cells having previously been infected with a recombinant defective adenovirus according to the invention.

The properties of some of these cytokines are recalled below.

Interleukin-1 (IL-1):

This is produced essentially by activated macrophages and monocytes. Its molecular weight is approximately 17 kilodaltons. It displays several activities, including:

a) a chemoattractive action on polymorphonuclear cells and macrophages (1, 2),

b) an increase in the cytotoxic activity of spontaneous cytotoxic (natural killer or Nk cells),

c) an induction of fever following an infection,

d) most especially, the activation of T cells for the production of other factors.

Interleukin-2 (IL-2), Interleukin-4 (IL-4) and Interleukin-5 (IL-5):

These are produced by activated T lymphocytes. The action of these cytokines is restricted to cells of the immune system, they cause their multiplication and their activation: IL-2 and IL-4 have been tested in antitumor immunotherapy in mice and in man. In mice, they act synergistically and cause tumor regression.

Interleukin-6 (IL-6)

This is produced by many cell types including T lymphocytes, macrophages, fibroblasts, etc. It induces the final differentiation of B lymphocytes, which become antibody producers.

Tumor Necrosis Factor α (TNFα) ([same term in French]):

This is a factor produced by macrophages. It has a dual action: a direct action on tumor cells causing their lysis and an activation of the immune system.

The use of TNFα in man must be carried out cautiously since a large number of cells possess the receptor for it: which accounts for the importance of inducing its secretion only locally, actually within the tumor, to limit its adverse effects on the other cells of the host.

Interleukin-3- (IL-3), Interleukin-7 (IL-7) and Colony stimulating Factor (CSF).

These are hematopoietic growth factors. They are produced essentially by lymphocytes, monocytes and macrophages. They act at different levels of hematopoiesis, that is to say of the different stages of

differentiation of marrow cells to blood cells. In addition, CSF exerts very substantial effects on the body's primary defences as regards the bacterial defences, luring the macrophages to the sites of infection and increasing their capacity for phagocytosis.

In combination with IL-2 and IL-4, GM-CSF proves to be an important antitumor factor.

γ Interferon (IFN-γ)

This is a factor produced by activated T cells; it is endowed with antiviral properties; it inhibits the multiplication of viruses and parasites and causes the lysis of infected cells and some tumor cells.

α Interferon (IFN-α)

Produced by T cells and monocytes, this displays an antiviral and lytic effect on infected cells. IFN-α has been used in immunotherapy against some types of cancer, including mesothelium [sic].

Naturally, the invention is not limited, as regards the choice of insertion sequences which can be used in adenoviruses according to the invention, to those sequences which have been identified above. Nevertheless, the latter are illustrative of the palette of possibilities which are afforded to the therapist, who is responsible for making the choice of the most suitable defective recombinant adenovirus to be used in the light of the nature of the tumours to be combated.

The invention also relates to pharmaceutical compositions comprising one or more recombinant vectors as described above, in combination with a

pharmaceutically acceptable vehicle, especially sterile, isotonic compositions which can be injected directly into the tumors to be treated, or dry, in particular lyophilized, compositions which, by the addition of sterilized water or of physiological saline as the case may be, enable solutions which can be injected directly into the tumors to be made up or reconstituted.

Direct injection of non-replicable, modified adenoviruses into the tumor affords the advantage, on the one hand of avoiding diffusion of the recombinant adenoviruses in the general circulation, with the consequent side effects liable to be exerted by the cytokines expressed in places other than on the sites where the manifestation of their action is sought, in this instance the tumor cells themselves or the cells, in particular lymphocytes, which infiltrate them or which are present in their immediate proximity. Preferably, the injection is carried out at the very least in at least one site of the primary tumor.

Neither is the invention limited to administration of the recombinant adenoviruses containing the sequences coding for the cytokines of the kind in question directly in the tumors. Any other administration route permitting access of these recombinant adenoviruses to the tumor to be treated may be envisaged. In particular, use may be made of cells which are compatible with the host, for example human fibroblasts, preferably ones previously removed from the host him- or herself.

The invention also relates to cell cultures, for

example cultured fibroblasts previously infected with recombinant nucleic acids, more especially the defective adenoviruses defined above. These infected cells, where appropriate attenuated or rendered immunologically inert, for example by irradiation, contribute to the eradication of installed tumors when they are injected systemically. This injection may be envisaged either alone, or in addition to injection directly into the tumor.

The subject of the invention is also a method for obtaining the recombinant adenoviruses described above, which comprises, after the actual step of construction of a vector by introduction of one or more insertion nucleic acid(s) into the genome of the initial defective adenovirus, a step of transformation of transformable cell lines of higher eukaryotes (in particular of human or animal origin) themselves containing a separate nucleotide sequence capable of complementing the portion(s) lacking in the genome of the defective adenovirus and without which replication of the latter is prevented, said separate sequence preferably being incorporated in the genome of the cells of said cell line.

As a preferred example of such cell lines, there may be mentioned line 293, a human embryonic kidney line which contains, integrated in its genome, the first eleven percent of the left-hand end of the genome of a type 5 adenovirus (Ad5). This fraction can then complement defective recombinant viruses which carry deletions of this region. Such a production method is described, more especially, in European Patent Application

No. 0,185,573 of 20/11/85.

After transformation of these cell lines, the defective adenoviruses thus multiplied and produced are recovered from the culture medium of the cells of these lines and purified.

Further details of the present invention will be given in the description which follows of the possibilities of construction of a recombinant vector adenovirus containing at least one sequence coding for a cytokine, especially a lymphokine.

I. METHODS

A) Cells and viruses

Ad-5-transformed human embryonic kidney cell line 293 (Graham et al., 1977) was used for the transfection of DNA as well as for the multiplication and titration of adenovirus (Ad). In effect, cell line 293 complements the functions of the genes for E1A and E1B functions and permits the replication of defective Ad recombinants. For the construction of the recombinant Ad, human Ad5-d1324, carrying deletions in the E1 region (3.9-10.5 m.u.) and E3 region (78.5-84.3 m.u.), was used (Shenk and Williams, 1984). Cell lines 293, Hela [sic] and Vero were maintained in an Eagle minimum essential culture medium with 10% of fetal calf serum.

b) Construction of plasmids permitting the expression of different cytokines

The eukaryotic expression vector pMLP10 has been described (Ballay et al., 1985). A derivative of this vector (pMLP18) was constructed by insertion of a

sequence containing different single restriction sites downstream of the adenovirus major late promoter. These sites thus permit cloning of the different genes coding for the chosen cytokines under the control of the viral promoter. Downstream of this sequence containing these single restriction sites, the sequence containing the polyadenylation signal of the gene coding for the early antigens of SV40 virus were placed. The BgIII - HindIII fragment of Ad5 is cloned downstream. This 3-Kbp sequence contains the gene coding for protein IX which is necessary for encapsidation of the viral genome exceeding 97% of its normal size, and permits subsequent in vivo recombination. Sequences coding for the genes for the different cytokines are isolated from plasmids obtained from different teams. These sequences, obtained after cleavage by means of different restriction enzymes, are introduced into the multiple cloning site of the expression vector described above (pMLP-18). The different plasmids designated pMLP-cytokine (IL-2, IL-4, and the like), which are used for obtaining the recombinant viruses as described in the following section, are thereby obtained.

c) Transfection of DNA and isolation of recombinant viruses

The Ad-cytokine defective recombinant adenoviruses were obtained by in vivo recombination between the straight fragment of the viral genome previously cleaved with the restriction enzyme Cla I and the homologous sequence existing on the plasmids

pMLP-cytokine described above. The mixture of the fragment of the viral genome (2.6 m.u. - 100 m.u.), purified after cleavage, and of the plasmid linearized with the restriction enzyme Cla I or Pvu I is transfected into 293 cells using the calcium phosphate precipitation method (Graham and Van der Eb, 1973). Cell plaques showing a cytopathic effect are isolated 10 days later and the virus was amplified in culture. The viral DNA was extracted by the Hirt procedure (Graham et al., 1977) and the recombinant viruses were identified by mapping with restriction enzymes.

Figure 1 shows diagrammatically a construction of this type employing an insertion sequence coding for an interleukin (IL-2, IL-4, and the like).

In this figure:

- "leaders" corresponds to a tripartite leader
- "Del" corresponds to a "deletion"
- Ad dl 324 corresponds to an adenovirus provided with the abovementioned "deletions".

d) <u>Expression of the sequences coding for an expressed cytokine</u>

Hela or Vero cell lines are infected with the defective recombinant viruses obtained. Cells effectively transfected may be characterized essentially by means of detection of the activity of the cytokine released into their culture medium. In the case of IL-I, yields capable of reaching from 1 to 2 μg of interleukin per 10^6 cells have been observed.

Cells infected with an Ad-cytokine recombinant

secrete variable amounts of the cytokine into the culture medium. Different methods exist for the detection and quantification of the cytokines produced.

1) Quantitative methods:

- ELISA, using specific antibodies
- RIA (radioimmunoassay)
- Western blotting

2) Qualitative methods (or biotests): based on the biological properties of cytokines

For example:

IL-2: Test of proliferation of CTL-L2 cells (CTL-L2 cells multiply and are maintained in culture only in the presence of IL-2 in the culture medium)

IL-3 and GM-CSF: Test of proliferation of TF-1 cells

IL-4: Test of proliferation of CTL-L2 cells and induction of soluble CD23 with certain cells including lymphocytes.

INF-α: Cytotoxicity test on L92-9 cells.

Neutralization test: The effect of cytokines may be blocked by incubating the target cells in the presence of cells of specific antibodies.

Some results obtained with the adenovirus vector carrying the IL-2 gene (Ad-IL-2) are described below.

1) Cells infected in vitro with Ad-IL-2 secrete significant amounts of functional IL-2.

2) Direct injection of the vector carrying the IL-2 gene into tumors already established in the animal (the tumor diameter at the time of injection is between 4 and

7 mm) induces stimulation of the immune system which manifests itself in a stabilization of the size of the tumor, and then its regression to the point of complete disappearance in 40% to 50% of cases.

This result may be improved by treating the tumors in an earlier phase of its development or by using different vectors at the same time, for example combination of Ad-IL-2 with Ad-INF and/or Ad-IL-4, Ad-GM-CSF, Ad-IL-3. This combination has to be specified according to the type of tumor.

3) Tumor cells infected in vitro and then injected into syngeneic animals or even immunodeficient animals (Nu/Nu mice) lose their tumorigenic power (at least up to 80% of the animals reject the tumor cells; in other words, the tumor cells no longer proliferate in 80% of immunodeficient animals injected with these cells.

4) Animals which have rejected a first injection of infected tumor cells are highly immunized and are protected against parent (uninfected) tumor cells injected at different times and at different places.

When coinjected with tumor cells infected in vitro, the spleen cells of these immunized animals are, furthermore, capable of transferring the antitumor immunity to recipient animals.

It is self-evident that the descriptions of constructions of recombinant defective adenoviruses envisaged above are in no way limiting in character. Other constructions may be produced, in particular

according to the variants also mentioned below as examples.

1) Promoter exchange

The adenovirus major late promoter may be replaced by other promoters which are ubiquitous but of exogenous origin, such as:

- promoter contained in the LTR (long terminal repeat) of RSV (Rouse sarcoma virus)
- the promoter of the IE gene of CMV (cytomegalovirus)
- The MMTV (mouse mammary tumor virus) or metallothionine inducible promoters.

Similarly, promoters permitting a more specific expression restricted to tumor cells, may be used, such as, for example:

- the promoter of the rep gene of parvovirus HI.

The invention also relates to a recombinant nucleic acid of the abovementioned type, characterized in that the genomic sequence of the adenovirus lacks its 5' end region downstream of the early promoter of the E1A region of the adenovirus, and in that the sequence coding for the cytokine is placed under the control of this early promoter. This recombinant nucleic acid may also be employed in applications mentioned more especially in connection with recombinant DNAs in which the sequence coding for the cytokine is placed under the control of the adenovirus major late promoter.

2) Simultaneous expression of several cytokine genes

3 types of constructions are described:

- the cytokine genes are under the control of two promoters which are either identical or different (MLP and RSV, for example) and are located following one another.
- the cytokine genes are under the control of separate promoters cloned into separate regions of the virus.

CLAIMS

1. Recombinant nucleic acid containing, on the one hand a genomic sequence of an adenovirus which is defective in that it lacks the sequences needed for its replication, but which nevertheless contains those sequences which, in this genome, are the carrier of the genetic information needed for the corresponding adenovirus to enter the cells which the latter is capable of infecting, as well as the set of essential sequences needed for encapsidation of this adenovirus, and on the other hand an insert containing a nucleic acid sequence coding for a cytokine, this insert being under the control of a promoter present in or previously inserted into the abovementioned genomic sequence.

2. Recombinant nucleic acid according to claim 1, characterized in that it lacks the transactivators E1A and E1B and, where appropriate, the E3 region of the adenovirus.

3. Recombinant nucleic acid according to claim 1, characterized in that the genomic sequence of the adenovirus lacks its 5' end region downstream of the early promoter of the E1A region of the adenovirus, and in that sequence coding for the cytokine is placed under the control of this early promoter.

4. Recombinant nucleic acid according to claim 1 or claim 2, characterized in that the sequence coding for the cytokine is placed under the control of an adenovirus late promoter.

5. Recombinant nucleic acid according to claim 1,

characterized in that the genomic sequence of the adenovirus is provided with a promoter foreign to the adenovirus genome, and in that the sequence coding for the cytokine is placed under the control of this foreign promoter.

6. Recombinant nucleic acid, characterized, either in that the insert contains sequences coding for several cytokines, or in that it contains separate inserts placed, respectively, under the control of separate promoters, which are also separate.

7. Defective adenovirus, characterized in that it contains recombinant nucleic acid according to any one of claims 1 to 6.

8. Culture of cells, in particular of human origin, characterized in that they are infected with the adenovirus according to claim 7.

9. Pharmaceutical composition containing the recombinant adenovirus according to claim 7, in combination with a pharmaceutically acceptable vehicle, in particular.

10. Use of the recombinant adenovirus according to claim 7 for the preparation of antitumor drugs, preferably in a form which can be injected directly into a tumor of the host.

11. Pharmaceutical composition containing cells according to claim 8, preferably human cells, in a state allowing them to be injected into humans.

12. Method for the production of recombinant defective adenoviruses according to claim 7,

characterized by the transformation of transformable cell lines of higher eukaryotes (in particular of human or animal origin) themselves containing a separate nucleotide sequence capable of complementing the portion of the adenovirus genome which is lacking in the adenovirus and which would be essential for its replication, said separate sequence preferably being incorporated into the genome of the cells of said cell line, and in that the defective recombinant adenoviruses produced are recovered from the culture medium of the cells of said cell lines.

13. Method according to claim 12, characterized in that the genome of the defective adenoviruses lacks its 5′ end region, and in that the cell line is a human embryonic kidney line such as line 293 which contains, integrated in its genome, a 5′ end region of the genome of a type 5 adenovirus (Ad5) and having a size corresponding to approximately 11% of that of the whole genome of this adenovirus.

Abstract

Recombinant nucleic acid for use in the production of a defective adenovirus containing an inserted sequence coding for a cytokine under the control of a promotor in the genomic sequence of the recombinant adenovirus. Said recombinant adenovirus is useful in the preparation of anti-tumoral drugs capable of being directly injected into the tumour of the host.

PATENT
Attorney Docket No.8076.102USF2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)
)
HADDADA et al)
) Group Art Unit: 1632
Serial No.:08/619,157)
) Examiner: S. Priebe
Filed: March 21, 1996)

For: DEFECTIVE RECOMBINANT ADENOVIRUSES EXPRESSING CYTOKINES FOR USE IN ANTITUMORAL TREATMENT

DECLARATION PURSUANT TO 37 C.F.R. §1.132

I, Majid Mehtali, do hereby declare and state the following:

1.) That I have received an Engineer Diploma in Biotechnology in 1985 from the European School of Biotechnology of the Upper Rhine Region, Strasbourg, France. In 1988, I received a Ph.D. in Molecular Biology at the Institute of Molecular Genetics at the University of Strasbourg in France.

2.) In 1984 I worked for three months at Roche in the laboratory of Dr. R. Then and in 1985 I worked nine months at Rhone-Merieux in Lyon, France in the laboratory of Dr. G. Chappuis. I have been employed at Transgene S.A. since 1988 and I currently head the Gene Therapy Department at Transgene S.A. Enclosed, please find a copy of my *Curriculum vitae*.

3.) I have read and understood the above-captioned patent application, as well as the pending claims of record. I have also read and understood the latest Official Action issued by the U.S. Patent and Trademark Office on February 3, 1998.

It is my understanding that Claims 1 and 3 to 8 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Rosenfeld et al taken with Russell, Ramshaw et al and Stratford-Perricaudet et al. It is my understanding that this rejection means that the claims of record were obvious to the skilled scientist when their teachings are combined.

4.) I have reviewed the publications of Rosenfeld et al, Russell, Ramshaw et al and Stratford-Perricaudet et al that were cited in the above-mentioned Official Action. It is my opinion that a skilled scientist would not come to the same conclusions as the Examiner has for the following reasons.

5.) First of all, as I understand, the present invention relates to a defective adenoviral vector and an insert containing a nucleic acid sequence coding for a cytokine. The defective adenoviral vector lacks the transactivators E1A, E1B and the E3 region of the adenovirus. The defective adenoviral vector can be used to treat tumors. Although it was known prior to the filing of this application that cytokines have been used to treat tumors with some success via systemic injection, side effects such as fever, fluid retention and life-threatening vascular leak syndrome often resulted due to the high dosage given to the patient which was needed to treat the tumors. To solve this problem use of a different type of delivery system was necessary for making the tumor cell a source of cytokine production without severe toxicity.

6.) Prior to the filing date of the present application, which I understand is March 18, 1992, many vector systems were available for use to the skilled scientist. The different vector systems available then included vaccinia virus, pox virus, HTLV-I, retroviral vectors, herpes virus, bacterial vectors, human parovirus vectors such as human AAV and H1, as well as the mouse vector MVM p, synthetic vectors and adenoviral vectors. However, to the best of my knowledge there was no precise guidance given to the skilled scientists to make a particular selection among the known vectors to treat cancerous tumors. In fact, among the various known vectors there were often problems associated with their use. This, was for example, reviewed in the Russell reference, which is discussed below.

7.) It is my opinion that from the teachings of Russell, a skilled scientist would glean that this reference teaches against using a defective recombinant vector due to the problems associated with access by defective vectors to poorly vascularized tumor regions. This is clear from the teachings at page 198, first column.

Moreover, Russell recognizes the need to develop suitable vectors for gene delivery and expression, since in 1990 there were problems associated with the vector systems. However, there is no teaching in Russell concerning what vector systems would in fact work. The only guidance given to the skilled scientist in Russell was the recognition that competent viral vectors should be chosen since they could facilitate infection of a higher proportion of tumor cells.

8.) Ramshaw et al disclose a variety of vaccine vector systems such as poxvirus, ~~vaccinia virus~~, herpes virus, adenovirus or bacteria in which a nucleic acid encoding a lymphokine is disclosed. The vaccine vector systems described in this reference are competent and thus viable vectors. The reason why Ramshaw et al teach the use of viable vectors is to enhance the immune response to the antigenic polypeptide that is expressed, which can be a "native" sequence of the host vector itself. Therefore, the skilled scientist would not use defective vectors to accomplish the teachings of Ramshaw et al.

Moreover, the Examples clearly demonstrate that vaccinia virus was the vector of choice. Although Example 4 illustrates a competent adenoviral vector only lacking the E3 region it appears that this example is a mere afterthought.

9.) It is my understanding that the Examiner has relied on the teachings of Rosenfeld et al to encourage the use of adenoviral vectors in which the entire E1 region can be removed. More specifically, the Examiner deems that following teaching in Rosenfeld would encourage a skilled scientist to delete the E1 region:

> Most human adults have antibodies to one of the three serogroup C adenoviruses to which Ad5 belongs (5). This implies little risk to those

working with these vectors but may have negative implications for the virus as a gene transfer vector in the human lung. If such problems are encountered alteration in the vector construct may be helpful.

However, this paragraph cannot be interpreted as meaning that the E1 region should be deleted. Indeed, this paragraph means that most human adults have antibodies to adenoviruses and hence repeated administration of the adenoviral vector may result in the antibodies "killing" the administered adenovirus and therapy would not be effective. If this is the case, one would have to alter the vector such that the antibodies would not recognize the surface of the virus or the capsid. It should be noted that the E1 region is not within the capsid.

Moreover, the entire E1B region in the construct of Rosenfeld et al is maintained, as well as the 3' part of the E1A coding region from 936 to 1540 bp.

Thus, it is my opinion that a skilled scientist would not be guided to remove the E1A and E1B regions from the teachings of Rosenfeld et al. Moreover, this reference relates to defective gene therapy and not to treat tumors which is discussed more extensively under point 11 below.

10.) Stratford-Perricaudet et al teach the use of defective adenoviral vectors for selective delivery to certain tissues. It is clear to the skilled scientist that this publication is a general overview of the promising aspects of using adenoviral vector constructs for use in certain gene therapies such as OTC and other enzyme deficiencies directed to therapy of genetic diseases, which restores a defective function *in vivo*.

A skilled scientist would not know from reading this reference if an adenoviral vector can be used to treat cancerous tumors, since Stratford-Perricaudet et al teach replacement gene therapy.

11.) Cancer therapy is totally different from therapy that restores a defective gene. For example, recombinant cytokines were known to have a very short half-life

in vivo resulting in the necessity for continuous infusions or regular injections. The same is not true for many replacement therapies.

Secondly, local delivery of cytokines, and especially IL-2 had added difficulties of access to tumor deposits and is totally inadequate for occult metastic disease. This is a different situation from replacement gene therapies where certain tissues such as the lung lacking α-1AT, for example, could be targeted.

Thirdly, adenoviral vectors were known to be quite immunogenic; i.e., Rosenfeld et al recognized this problem. Although this immunogenicity may be a disadvantage for some gene therapies, it is beneficial for immunotherapy since this immunogenicity will limit the duration of cytokine expression and provide adjacent stimulous for the development of antitumor immunity.

In conclusion, it is my opinion that gene therapy to threat tumors is different from gene therapy to correct a deficient gene. Thus, a skilled scientist would not necessary interchange a "delivery system" for gene therapy of genetic diseases and cancer therapy without some suggestion or guidance given in the scientific literature that it is feasible.

12). I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Nov. 18, 1998
Date

Majid Mehtali, Ph.D.

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CURRICULUM VITAE

Majid MEHTALI, PhD

PERSONNAL

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date of birth	: March 14th, 1962

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EDUCATION :

High School, Saint-Louis, France
1980 : Baccalaureat D (Mathematics, Physics, Biology)

European School of Biotechnology of the Upper rhine Region, Strasbourg, France
1982-1985 : Engineer Diploma in Biotechnology

University of Strasbourg, France
1980-1982: Diploma of General Biological University Studies (DEUG. B)
1983: Licence in Biochemistry
1984: Maitrise in Biochemistry
1985: D.E.A. in Molecular Biology (equivalent to Msc)
1985-1988: PhD in Molecular Biology at the Institute of Molecular Genetics (Director: Pr. P. Chambon). Topic: *in vitro* and *in vivo* (in transgenic mice) analysis of the role of specific regulatory sequences from housekeeping genes

PROFESSIONAL EXPERIENCE :

1984: 3 months period at Roche (Basel) in the laboratory of Dr. R. Then (Pharmaceutical Research Dpt); topic: biochemical analysis of the bacterial porins isolated from antibiotic-resistent strains.

1985: 9 months period at Rhône-Mérieux Company (Lyon, France) in the laboratory of

Dr. G. Chappuis; topic: identification and biochemical characterization of the pathogenic agents (later shown to belong to the Pestiviruses virus family) responsible for bovine and porcine diseases.

1988: Staff Scientist at Transgene S.A.
Research projects:
(i) development of novel transgenic animal models (mice and rabbits) for the evaluatation of potential anti-HIV1 treatments and characterisation of the role of major HIV regulatory proteins in AIDS pathogenesis;
(ii) producion and evaluation in rhesus and cynomolgus macaques of various recombinant AIDS vaccine candidates (Live attenuated viruses, recombinant purified viral proteins, poxvirus-derived vaccines, pseudovirions,...).

1991-1992: Head of the Virology-Immunology department at Transgene S.A.
Research projects:
(i) development and evaluation of candidate AIDS vaccines;
(ii) development and evaluation of new immunotherapeutic approaches for breast cancer.

1992-1998: Head of the Gene Therapy department at Transgene S.A.
Research projects:
(i) development of novel generations of safer and more efficient viral (human and animal adenovirus, murine retrovirus, simian lentivirus) and cellular vectors for gene therapy;
(ii) development and evaluation *in vitro* and *in vivo* of gene therapy strategies for cancer, AIDS, Heamophilia and cardiovascular diseases;

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No :
U.S. National Serial No. :
Filed :
PCT International Application No. : PCT/FR93/00264

VERIFICATION OF A TRANSLATION

I, the below named translator, hereby declare that :
My name and post office address are as stated below ;
That I am knowledgeable in the French language in which the below identified international application was filed, and that, to the best of my knowledge and belief, the English translation of the international application No. PCT/FR93/00264 is a true and complete translation of the above identified international application as filed.
I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the patent application issued thereon.

Date :18 November 1993

P. A. Kendall

Full name of the translator : Philip Arnold KENDALL
For and on behalf of RWS Translations Ltd.

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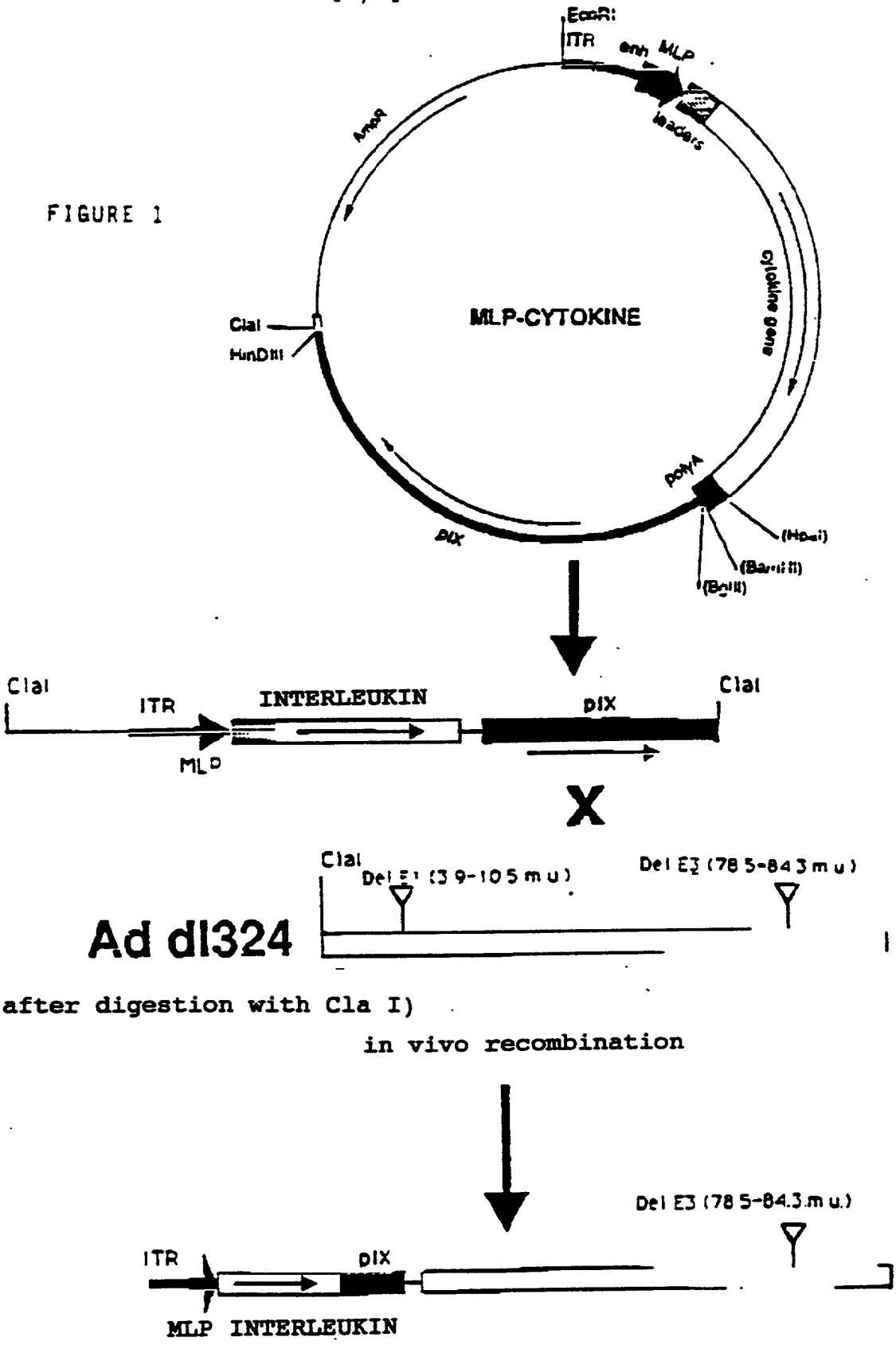
FIGURE 1


EcoRI
ITR
enh
MLP
leaders
AmpR
cytokine gene
MLP-CYTOKINE
ClaI
HindIII
polyA
pIX
(HpaI)
(BamHI)
(BglII)
ClaI
ITR
INTERLEUKIN
pIX
ClaI
MLP
X
ClaI
Del E1 (3 9-10 5 m u)
Del E3 (78 5-84 3 m u)
Ad dl324
(after digestion with Cla I)
in vivo recombination
Del E3 (78 5-84.3 m u.)
ITR
pIX
MLP INTERLEUKIN

For Utility, PCT, and Design Applications

MERCHANT & GOULD

United States Patent Application

▼ INSTRUCTIONS

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that

I verily believe I am the original, first and sole inventor (if only one name is listed below) or a joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Insert TITLE of invention

DEFECTIVE RECOMBINANT ADENOVIRUSES EXPRESSING CYTOKINES FOR ANTITUMOR TREATMENT

Check a or b

The specification of which

a. ☒ is attached hereto

b. ☐ was filed on November 10, 1993

If "b" checked, complete

as application serial no. 08/150011

and was amended on _____ (if applicable)

If PCT Application

(in the case of PCT-filed application)

Insert Int. application number & filing date

described and claimed in international no. PCT/FR 93/00264 filed March 16, 1993

and as amended on _____ (if any), which I have reviewed and for which I solicit a United States patent.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a). (Reprinted on back side).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119/365 of any foreign application(s) for patent of inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on the basis of which priority is claimed:

Prior applications
Check a or b

a. ☐ no such applications have been filed.

b. ☒ such applications have been filed as follows:

If "b" checked, complete

FOREIGN APPLICATION(S), IF ANY, CLAIMING PRIORITY UNDER 35 USC §119			
COUNTRY	APPLICATION NUMBER	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)
FRANCE	9203120	16/03/1992	
ALL FOREIGN APPLICATIONS, IF ANY, FILED BEFORE THE PRIORITY APPLICATION(S)			
COUNTRY	APPLICATION NUMBER	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)

I hereby claim the benefit under Title 35, United States Code, § 120/365 of any United States and PCT international application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application

	DATE OF FILING (day, month, year)	STATUS(patented, pending, abandoned)

I hereby appoint the following attorney(s) and/or patent agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected herewith:

Bartingale, Karl H	Reg. No. 35,183	Kluth, Daniel J	Reg No 32,146	Schwappach, Karl G	Reg No. 35 786
Batzli, Brian H.	Reg No 32, 960	Kowalchyk, Alan W	Reg. No 31,535	Schwegman, Micheal L	Reg. No 25 816
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Brennan, Thomas F	Reg No 35,075	Lynch, David W	Reg. No 36,204	Smith, Stephanie J.	Reg No. 34,437
Bruess, Steven C.	Reg. No. 34,130	Mau, Michael L.	Reg. No 30,087	Sorenson, Andrew D.	Reg No. 33,606
Byrne, Linda M.	Reg. No. 32,404	Maxin, John L.	Reg. No 34,668	Strawbridge, Douglas A.	Reg. No. 28,376
Carlson, Alan G.	Reg. No. 25,959	McDonald, Daniel W	Reg No. 32,044	Strodthoff, Kristine M.	Reg. No. 34,259
Caspers, Philip P	Reg. No 33,227	McDonald, Wendy M	Reg. No. 32,427	Sumner, John P.	Reg. No. 29,114
Clifford, John A.	Reg No 30,247	Michel, Michelle M	Reg. No. 33,968	Sumners, John S.	Reg. No. 24,216
Conrad, Timothy R	Reg. No. 30,164	Moy, R. Carl	Reg No 30,725	Tellekson, David K.	Reg. No 32,314
DiPietro, Mark J.	Reg No 28,707	Mueting, Ann M.	Reg No. 33,977	Underhill, Albert L.	Reg. No 27,403
Edell, Robert T.	Reg. No. 20,187	Mundelius, Anthony C	Reg. No 35,963	Vandenburgh, J Derek	Reg No 32,179
Freed, Robert C.	Reg No 32,569	Nelson, Albin J.	Reg No 28,650	Welter, Paul A.	Reg. No. 20,890
Gates, George H.	Reg No. 33,500	Raasch, Kevin W	Reg. No. 35,651	Williams, Douglas J.	Reg. No. 27,054
Golla, Charles E	Reg No 26,896	Reiland, Earl D.	Reg. No. 25,767	Woessner, Warren D	Reg. No 30,440
Gould, John D.	Reg. No 18,223	Rothfus, Joel A	Reg. No. 33,277	Wood, Gregory B	Reg No. 28,133
Gresens, John J.	Reg. No. 33,112	Schmidt, Cecil C	Reg. No. 20,566		
Hamre, Curtis B.	Reg. No. 29,165	Schuman, Mark D.	Reg. No. 31,197		
Hassing, Thomas A.	Reg. No. 36,159	Schumann, Michael D	Reg. No. 30,422		
Hillson, Randall A.	Reg. No. 31,838				

I hereby authorize them to act and rely on instructions from and communicate directly with the person/assignee/attorney/firm/organization/who/which first sends/sent this case to them and by whom/which I hereby declare that I have consented after full disclosure to be represented unless/until I instruct Merchant, Gould to the contrary.

Please direct all correspondence in this case to Merchant, Gould, Smith, Edell, Welter & Schmidt at the address indicated below (or if no address is specified, the first address):

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Telephone No. (612) 332-5300 Telephone No. (612) 298-1055

☐ Suite 1700, 11100 Santa Monica Boulevard, Los Angeles, CA 90025-3302
Telephone No. (310) 445-1140

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Insert FULL name(s) AND address(es) of actual inventor(s)

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	POST OFFICE ADDRESS	POST OFFICE ADDRESS: 1, rue Jules Guesde	CITY: ALFORTVILLE	STATE & ZIP CODE/COUNTRY: FRANCE
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	RESIDENCE & CITIZENSHIP	CITY: 92190 MEUDON	STATE OR FOREIGN COUNTRY: FRANCE	COUNTRY OF CITIZENSHIP: FRANCE
	POST OFFICE ADDRESS	POST OFFICE ADDRESS: 12A rue Charles Infroit	CITY: MEUDON	STATE & ZIP CODE/COUNTRY: FRANCE
203	FULL NAME OF INVENTOR	FAMILY NAME: PERRICAUDET	FIRST GIVEN NAME: Michel	SECOND GIVEN NAME:
	RESIDENCE & CITIZENSHIP	CITY: 28320 ECRIGNOLLES	STATE OR FOREIGN COUNTRY: FRANCE	COUNTRY OF CITIZENSHIP: FRANCE
	POST OFFICE ADDRESS	POST OFFICE ADDRESS: 31 rue de Chartres	CITY: ECRIGNOLLES	STATE & ZIP CODE/COUNTRY: FRANCE

Each inventor must sign & date

Note: No legalization or other witness required

SIGNATURE OF INVENTOR 201	SIGNATURE OF INVENTOR 202	SIGNATURE OF INVENTOR 203
DATE: DEC. 15, 1993	DATE: DEC. 15, 1993	DATE: DEC. 15, 1993

For Additional Inventors

☐ Check box and attach sheet with same information, including date and signature

Revised 11/1/92